CRDF Commercial Product Delivery Sub-Project Progress Report FY 2016-17

Quarter Ending December 30, 2016

1. *Candidatus Liberibacter asiaticus* PATHOGEN INTERVENTION

Project title: 1a. Bactericide Strategies

The goal of this project is to identify bactericides effective against Huanglongbing (HLB). Project managers will identify bactericides from various sources from products in the market to materials in early stages of development that are effective against HLB, and assist with formulation for effective delivery, provide regulatory guidance by engaging regulatory consultants and EPA and assist with commercialization if necessary. This is an ongoing project that will build on the development of an assay pipeline for screening bactericides and the *in vitro* screening of more than eight hundred compounds including material libraries from agriculture, biotech and pharmaceutical companies. Bactericides that have been identified by project managers, as potential short to long-term solutions will continue to be tested in assays and in field trials and steps will be taken to encourage commercialization of these materials to provide a solution to growers for HLB.

Subproject Title: 1aI. Bactericide Strategies: Candidate Bactericide Testing

Narrative of Progress against Goals:

Obj. 1 - Form relationships with companies with candidate bactericides for testing in the CRDF assay pipeline. Assemble data on potential bactericides to assist in prioritization.

CRDF is focusing on chemicals that can be available to growers in the near-term, although new active ingredients are tested in the pipeline when appropriate. No new relationships have been formed with companies with potential bactericidal chemicals in this quarter, but new materials from three companies did enter the pipeline.

CRDF and other stakeholders have been in contact with an agricultural chemical company to discuss the development of partnership. This company plans to develop a screening pipeline to test their chemicals against HLB. The chemicals to be tested will mainly be biopesticides and plant defense modulators. A presentation by the company will take place in January 2017 and a decision on the partnership will occur in February 2017.

Obj. 2 - Move bactericide candidates through assay pipeline to identify promising materials for field trials.

Twenty-six candidate bactericides were advanced through the in vitro assay this quarter, of these candidates, two were advanced to the greenhouse assay. These two materials would be considered biopesticides for registration.

Ten of the materials tested this quarter may be considered biopesticides, but this has not yet been determined by consultation with the EPA. Eleven materials would be considered conventional pesticides and are still in early stages of development.

Two biopesticides have been tested in the greenhouse assay this quarter. A conventional pesticide will be tested next quarter and two chemicals that are in early stages of development.

Subproject Title: 1aII. Bactericide Strategies: Bactericide Delivery

Narrative of Progress against Goals:

Obj. 1 - Coordinate with researchers, companies and other institutions to define formulations and delivery methods for field trials with minimal regulatory requirements.

A project was approved this quarter to test new adjuvants for the introduction of chemicals into citrus. The project will begin on February 1st 2017. These chemicals are in early stages of development, a time-to-market has not yet been established and will depend on efficacy. Project managers are working closely with this company to move this project forward efficiently.

Early results from the CRDF trunk injection trials and results from UF researcher trials are being discussed by project managers to determine if this method will be beneficial to growers. The requirements for this decision are that the methods sufficiently improve tree health and are economically viable. Sufficient improvement in tree health has not been observed by the methods tested, but other methods may have a more significant effect.

Obj. 2 - Track RMC and CPDC research projects relevant to the formulation and delivery of bactericides against HLB; integrate findings into project planning.

CRDF project managers are working to develop a project to test new equipment for chemical delivery, this project will be presented for funding consideration in the next quarter.

A project is in development with a University of Florida researcher to evaluate the hypothesis that thermotherapy increases the uptake of bactericides. This study will evaluate uptake in small greenhouse plants after heat treatment using biochemical analysis. If uptake is improved, a field trial will be developed to test uptake in field trees.

A CRDF funded project to evaluate methods of detecting bactericides in plant parts was initiated in July 2016. This project is on track, the techniques can be used to detect and quantify both oxytetracycline and streptomycin in a citrus leaf. This goal of this project is to evaluate movement of bactericides in citrus to help develop better methods of formulation and delivery.

Subproject Title: 1aIII. Bactericide Strategies: Bactericide Field Testing

Narrative of Progress against Goals:

Obj. 1 - Managing existing field trials including analyzing data, refining treatments and reporting progress to CPDC.

The field trial to evaluate trunk injections as an application method on Hamlins, project 15-048C, was set-up in February 2016. Data collection will be completed in the third quarter of FY 2016-2017 and a full report will be presented at the Commercial Product Delivery committee meeting in March and in third quarter reports.

The biopesticide field trial, project 15-049C, was set-up in late February on Hamlins. Data collection will be completed in the third quarter of FY 2016-2017 and a full report will be presented at the Commercial Product Delivery committee meeting in March and in third quarter reports.

Since March 2016, the bactericides Mycoshield, Fireline and Firewall have been available for use in Florida. CRDF has set-up nearly 70 field trials with growers to evaluate the efficacy of individual grower applications. Data being collected includes disease severity, bacterial titer/C_t values, fruit drop and yield. Initial PCR and disease severity data has been collected, fruit drop data are being collected on Hamlins and grapefruit and yield data has be collected on Hamlins and grapefruit for several trials. Valencia harvest data will be collected in the third and fourth quarter. Data from seven trials has been analyzed, including harvest data, but after eight months no significant differences in treatments has been found. Data will continue to be analyzed as it is collected. The majority of these trials are in Valencia blocks that began treatments after harvest in 2016, the data from these trials will be from a complete season of bactericide treatments. These trials will be analyzed when harvest data is collected.

Registrant trials funded by CRDF continue for a third year. In 2016-2017, these trials focus on the use of alternating applications of oxytetracycline and streptomycin (three applications of each active ingredient) and a mixture of the two active ingredients for use in grapefruit. Application timing include applications at even intervals throughout the year and applications grouped in the spring and fall. This project is on track, objectives for this quarter have been completed. A presentation on this project is scheduled for the Citrus Show in Fort Pierce in January 2017.

Obj. 2 - Develop new field trials to test promising bactericidal therapies.

A field trial is in development to evaluate a combination therapy of bactericides and thermotherapy. This trial will be conducted in cooperation with a grower and a thermotherapy company. A second field trial based on the results of greenhouse study to investigate the rate of uptake of bactericides after heat treatment may be developed if improved uptake is confirmed.

Obj. 3 - Provide communication of progress towards project goals and results to CPDC, CRDF and growers.

A request recertification of the Tree Health Section 18 has been sent to the EPA Emergency Response Team from the Florida Department of Agriculture and Consumer Services. This request is for continued use of oxytetracycline and streptomycin in Florida in 2017. The current Section 18 expires on December 31, 2016.

CRDF Commercial Product Delivery Sub-Project Progress Report FY 2016-17

Quarter Ending December 31, 2016

1. Candidatus Liberibacter asiaticus PATHOGEN INTERVENTION

Project Title: 1b. Thermal Therapy to Reduce CLas Titer in Infected Trees

Project goal(s) for this project area for the next year:

1. Track ongoing research on thermal therapy and its role in HLB and tree health
2. Determine impact of thermal treatment on CLas acquisition by ACP.
3. Evaluate HLB infected citrus trees before and after thermal therapy treatments to encourage scale-up of individual tree, over-the row and root supplemental heat and evaluation of their performance in reducing disease and improving health of treated trees.
4. Continue outreach efforts to inform growers of optimized thermal treatments including CRDF sponsored field days to include thermal therapy researchers and active steaming commercial companies.

Narrative of Progress by Project Goals:

2. Determine impact of thermal treatment on CLas acquisition by ACP. Ongoing CRDF-funded research at UF and USDA does not identify how thermal treatment affects availability of CLas to be acquired by ACP feeding on treated trees. Discussions occurred on the need for this to be included in the MAC funding proposal addressing thermal therapy scale-up and research. Overlay of CLas acquisition testing on current field trials was suggested as a simple way to accomplish this goal. A project plan was developed by Kirsten Pelz-Stelinski of UF, IFAS, CREC and has been approved by CRDF, and subsequently approved for funding through the USDA MAC HLB program. This one-year research project has been completed and publication of the results are pending.

Obstacles: None for this period. All activities followed prescribed plans.

3. Refine requirements and environmental conditions for most effective thermal treatment.
The USDA, APHIS MAC group was charged to manage the federal funding to put HLB solutions in the hands of growers. This group quickly identified thermal therapy as a “shovel-ready” project area and encouraged development of project ideas and mechanisms to attract and encourage solvers to come forward with plans for scale-up, and to propose how this funding could facilitate rapid scale-up.

USDA, APHIS responded with consideration of a mechanism that has been used by their agency previously in seeking solutions to challenges, and plans were established to solicit solvers for thermal therapy scale-up. Two Mac projects were approved to facilitate scale-up and both were in place at the end of this quarter. Evaluation of thermal therapy conducted by those involved in scale-up is being initiated by the CRDF evaluation team. Six enterprises are either field testing machines in Florida or will have machines ready for testing or will have them field-ready within the next couple of months. Those with capability are operating at multiple locations in Florida, and the evaluation team is in the field conducting the evaluations.

CRDF CPDC moved forward with plans to coordinate evaluation efforts of thermal therapy. Building on the methods used to evaluate effects of other treatments (antimicrobials, soil amendments, etc.) on CLas and/or HLB and tree response, a before and after protocol was developed to document tree and environmental conditions surrounding thermal treatments and a data plan for follow-up so that individual trials will be evaluated similarly and treatments can be compared. This protocol has publicized on the CRDF web page so growers can do some self-assessments of their own thermal therapy trials and been implemented on a small scale with grower and research trials. The protocol will become standard in the MAC funded CRDF project to evaluate thermal therapy scale-up described above. An overview of current field activity that the CRDF evaluation team is engaged in follows:

3. Encourage scale-up of individual tree, over-the row and root supplemental heat and evaluation of their performance in reducing disease and improving health of treated trees.

Most trees being evaluated are in varying stages of the decline due to HLB; most are heavily managed for psyllid control, nutrient applications, root health, etc. Evaluation of thermal therapy conducted by those involved in scale-up is ongoing by the CRDF evaluation team. Six enterprises are operating field thermotherapy machines in Florida. At least two other companies are supported by USDA, APHIS, MAC to deliver additional thermal therapy to Florida for field trials. Those with capability are operating at multiple locations in Florida, and the evaluation team is in the field conducting the evaluations.

At this reporting period, 11 trials are being evaluated, with varying intensity and with different machinery delivering a range of temperature/duration combinations. Since the trials continue to be set up as opportunities arise, we are providing the current data sets associated with trials currently being conducted. None of these trials are completed, but the results to date provide a glimpse of the variation of measures and tree responses. Significant additional data analyses will be available following the 2015-16 fruit harvest, providing yield, quality and other metrics.

All of these trials have been subjected to the protocol for evaluation as outlined per the approved work plan. The CRDF evaluation team is working with commercial scale-up thermal treatment applicators, helping to lay out field trials, collecting pre-treatment PCR bacterial measures, and other parameters. According to the protocol, periodic data collection following treatments will assess the tree health response as well as the specific impact on CLas bacteria. Having 11 locations under evaluation is ahead of the plans, and we anticipate being able to conduct additional evaluations as others get dropped to fulfill the work plan and budget.

Additional trial evaluations have been established as thermal therapy providers are ready for evaluation of their machinery and treatments. Additional treatment sites have been established to evaluate the new generation machines from Dr. Ehsani (UF, IFAS), Premier Energy, and Daniel Scott.

Status at end of 24 months of the scale-up program:

While this project does not control the tempo of innovation or the timetables for the various solvers who are commercializing thermal therapy for HLB-infected trees in Florida, there is significant progress being demonstrated on several fronts that is driving the evaluation component of this project. Several participants have revised designs in response to early evaluation results, and have deployed next generation machines.

The 11 sites reported on in this period are all ongoing thermotherapy projects where tree responses to different thermotherapy conditions are being monitored. Some treated trees displayed previous short-term responses that have since disappeared. The different sites are of various aged trees and varieties. Most projects have recent post-treatment leaf samples awaiting PCR analyses. All data and observations should be considered preliminary, as monitoring tree status and data analysis are continuing.

Conserve Trial 1

On 2-26-16, 15 sets of paired uniform of trees of Valencia on Swingle rootstock were selected for evaluation. Fifteen trees were non-steamed control trees and 15 trees were steamed at 131 F for 30 s. By 12-30-16, average canopy volumes (CV, m^3), tree height (TH, m), trunk cross sectional area (TCSA, cm^2), Disease index (DI, 0-40) and fruit drop (Fr Dr) were all not affected by the treatment. Thus, all trees have recovered from the previous treatment 10 months ago and any short-term differences have disappeared. Data from June and Sept from previous reports have been included for reference. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Control

DATE	N	CV (m^3)	TH (m)	TCSA	DI (0-40)	Fr Dr
6-3-16	15	4.7	1.9	33.6	23.8	-
9-16-16	15	5.3	1.9	33.7	20.5	-
12-30-16	15	5.1	1.97	35.7	22.3	10.3

Treatment = 131 F 30 s

DATE	N	CV (m^3)	TH (m)	TCSA	DI	Fr Dr
6-3-16	15	4.9	2.0	32.3	23.9	-
9-16-16	15	5.3	2.0	33.3	20.6	-
12-30-16	15	5.0	1.98	34.4	21.4	7.6

Conserve Trial 2

On 3-2-16, 15 sets of paired uniform of trees of Hamlin on Swingle rootstock were selected for evaluation. Fifteen trees were non-steamed control trees and 15 trees were steamed at 131 F for 30 s. On 12-30-16, Canopy Volumes (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index (DI) were not affected by the treatment. Thus, all trees have recovered from the previous treatment 9 months ago and any short-term differences have disappeared. Data from June and Sept from previous reports have been included for reference. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Control

DATE	N	CV (m^3)	TH (m)	TCSA (cm^2)	DI (0-40)
6-3-16	15	4.5	1.9	27.3	23.1
9-16-16	15	5.4	2.0	29.1	18.5
12-30-16	15	5.9	2.1	29.8	22.2

Treatment = 131 F 30 s

DATE	N	CV (m^3)	TH (m)	TCSA	DI
6-3-16	15	4.5	2.0	26.4	23.1
9-16-16	15	5.3	2.0	28.8	18.9
12-20-16	15	6.1	2.1	29.9	21.8

Conserve Trial 3

On 2-26-16, 10 sets of 3 uniform trees of Valencia on Swingle rootstock were selected for evaluation. Ten trees were non-steamed control trees, 10 trees were steamed at 131 F for 30 s., and 10 steamed at 120 F for 40 s. On 9-16-16, and 12-30-16, canopy volumes (CV), tree height (TH), trunk cross sectional area (TCSA)and Disease index were evaluated. There were no treatment effects on these variables. Thus, all trees have recovered from the previous treatment 10 months ago and any short-term differences have disappeared. Data from Sept from previous reports have been included for reference. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Canopy Volume (m^3) 9-16-16

DMRT	CV (m^3)	N	Treatment
A	8.0	10	120 F 40 s
A	8.0	10	131 F 30 s
A	7.9	10	Control

Canopy Volume (m^3) 12-30-16

DMRT	CV (m^3)	N	Treatment
A	7.2	10	120 F 40 s
A	7.2	10	131 F 30 s
A	7.1	10	Control

Tree Height (m) 9-16-16

DMRT	TH (m)	N	Treatment
A	2.2	10	Control
A	2.2	10	131 F 30 s
A	2.1	10	120 F 40 s

Tree Height (m) 12-30-16

DMRT	TH (m)	N	Treatment
A	2.2	10	Control
A	2.2	10	120 F 40 s
A	2.1	10	131 F 30 s

Disease index (DI) 9-16-16

DMRT	DI	N	Treatment
A	17.0	10	120 F 40 s
A	16.6	10	Control
A	16.5	10	131 F 30 s

Disease index (DI) 12-30-16

DMRT	DI	N	Treatment
A	20.2	10	120 F 40 s
A	20	10	131 F 30 s
A	20	10	Control

Cutrale Trial 1. Discontinued.

Cutrale Trial 2

There were 10 sets of paired uniform 2 year-old Valencia trees on Swingle rootstock that were selected for evaluation. Ten trees were left as non-steamed control trees and 10 trees were steamed at 127 F for 90 s. On 10-18-16, canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA) and fruit drop (Fr Dr) were not affected by treatment. Treatment trees had a higher DI than untreated control trees. There were no treatment effects on CT values (previous report). Thus, all trees have recovered from the previous stream treatment and any short-term differences have disappeared. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Untreated Control **TCSA**

DATE	N	CV (m^3)	TH (m)	(cm^2)	DI (0-40)	Fr Dr
10-18-16	10	2.4	1.5	17.1	4.6 b	2.1

Treatment = 127 F 90 s

DATE	N	CV (m^3)	TH	TCSA	DI	Fr Dr
10-18-16	10	2.6	1.6	18.8	5.3 a	1.7

Cutrale Trial 3

There were 10 sets of paired uniform trees of 4 year-old Hamlin on Swingle rootstock selected for evaluation. Ten trees were non-steamed control trees and 10 trees were steamed at 127 F for 90 s. On 10-18-16, Canopy Volume (CV), tree height (TH), trunk cross sectional area (TCSA) and fruit drop (Fr Dr) were not affected by treatment. Treatment trees had a lower Disease index (DI) than untreated control trees. There were no treatment effects on CT (previous report). Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Treatment = Control **TCSA**

DATE	N	CV (m^3)	TH (m)	(cm^2)	DI (0-40)	Ft No.
10-18-16	10	6.8	2.3	36.2	15 a	55

Treatment = 127 F 90 s

DATE	N	CV	TH	TCSA	DI	Ft No.
10-18-16	10	7.0	2.3	36.5	14.3 b	80

Blue Goose Trial 1

There were 10 pairs of uniform trees selected for evaluation, 10 steamed and 10 non-steamed control trees. On 10-14-16, Canopy Volumes (CV), tree height (TH), trunk cross sectional area (TCSA), Disease index (DI) and fruit drop (Fr Dr) were evaluated. Canopy volume, TH and TCSA of the treatment trees were larger than the untreated control trees but DI and Fr Dr did not differ. CT values from PCR did not differ on 8-1-16 (previous report). Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Untreated Control **TCSA**

DATE	N	CV (m^3)	TH (m)	(cm^2)	DI (0-40)	Fr Dr
10-14-16	10	7.6 b	2.2 b	57.4 b	17.7	22.4

Treatment = 128 F 30 s

DATE	N	CV	TH	TCSA	DI	Fr Dr
10-14-16	10	9.2 a	2.4 a	68.2 a	16.8	28.9

Blue Goose Trial 2

There were 10 pairs of uniform trees selected for evaluation, 10 steamed and 10 non-steamed control trees. On 10-16-16, canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA), Disease index (DI) and fruit drop (Fr Dr) were evaluated. There were no significant treatment effects on any of the measured variables. Leaves were sampled for PCR on 8-1-16 but revealed no treatment effects on CT (previous report). Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Nontreated Control **TCSA**

DATE	N	CV (m^3)	TH (m)	(cm^2)	DI (0-40)	Fr Dr
10-14-16	10	7.6	2.1	55.9	23.2	26.1

Treatment = 128 F 30 s

DATE	N	CV	TH	TCSA	DI	Fr Dr
10-14-16	10	8.0	2.1	56.8	21.7	22

Blue Goose Trial 3

There were 10 pairs of uniform trees selected for evaluation, 10 steamed and 10 non-steamed control trees. On 10-14-16, Canopy Volumes, tree height, trunk cross sectional area and Disease index were evaluated. Tree height (TH), trunk XS area (TCSA) and fruit drop (Fr Dr) of treated trees were lower than the untreated control trees. Leaves were sampled for PCR on 8-1-16; there was no difference in CT between the treated trees and the untreated control trees (in previous report). Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Untreated Control **TCSA**

DATE	N	CV (m^3)	TH (m)	(cm^2)	DI (0-40)	Fr Dr
10-14-16	10	9.3	2.3 a	64.7 a	23.3	26.2 a

Treatment = 127 F 90 s

DATE	N	CV (m^3)	TH	TCSA	DI	Fr Dr
10-14-16	10	8.8	2.1 b	56.6 b	22.8	16.5 b

Scott Trial 3

Ray Ruby GF on Sour orange trees, 5 years old. On 6/30/15 and 20 trees were steam treated at either at 130 F for 15 s or at 130 F for 30 s. There were 15 non-treated control trees, resulting in the 3 treatments in this trial. On 10-11-16, Canopy Volumes (CV), tree height (TH), trunk cross sectional area TCSA), Disease index (DI), Yield (boxes) and average fruit weight (g) were not affected by treatment. Thus, all trees have recovered from the previous treatment 4 months ago and any short-term differences had disappeared. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

CV (m^3) 10-11-16

DMRT	CV	N	Treatment
A	12.1	12	130 F 30 s
A	11.6	15	Untreated
A	10.6	8	130 F 15 s

TH (m) 10-11-16

DMRT	TH	N	Treatment
A	2.28	15	Untreated
A	2.23	12	130 F 30 s
A	2.22	8	130 F 15 s

DI (0-40) 10-11-16

DMRT	DI	N	Treatment
A	19.9	15	Untreated
A	19.8	12	130 F 30 s
A	19.3	8	130 F 15 s

TCSA (cm^2) 10-11-16

DMRT	TCSA	N	Treatment
A	87.2	12	130 F 30 s
A	81.4	15	Untreated
A	77.0	8	130 F 15 s

Fruit Yield (boxes)

DMRT	Boxes	N	Treatment
A	1.7	12	130 F 30 s
A	1.4	13	Untreated
A	1.3	8	130 F 15 s

Avg Fruit weight (g)

DMRT	Fr Wt	N	Treatment
A	288	8	130 F 15 s
A	286	12	Untreated
A	266	12	130 F 30 s

Scott Trial 4

Ray Ruby Grapefruit on Sour orange trees, 6 years old. 12 trees each were steam treated on 9/8/15 at either 128 F for 120 s, 132 F for 1 s (turned off immediately when temperature in canopy reached 132 degrees, or at 132 F for 10 s. 12 trees were left untreated as a control. On 10-4-16, Canopy Volume (CV) of the untreated were larger than the 3 sets of steam treated trees so the treated trees suffered some canopy loss. The untreated control trees had a lower Disease index (DI) than the 128 F 20 s treated trees. Tree height (TH) was not affected by treatments. Fruit drop (Fr Dr) on 10-5-16 was lowest in the untreated control trees and highest in the 128 F 20 s treated trees. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Canopy Volume (m^3) 10-4-16

DMRT	CV	N	Treatment
A	24.3	12	Untreated
B	18.4	12	132 F 10 s
B	18.2	12	132 F 1 s
B	17.6	12	128 F 20 s

Tree Height (m) 10-4-16

DMRT	TH	N	Treatment
A	2.84	12	Untreated
A	2.62	12	128 F 20 s
A	2.58	12	132 F 1 s
A	2.58	12	132 F 10 s

Disease Index (0-40) 10-4-16

DMRT	DI	N	Treatment
A	23.8	12	128 F 20 s
A B	22.4	12	132 F 1s
A B	22.3	12	132 F 10 s
B	20.9	12	Untreated

Fruit drop (Fr Dr) 10-5-16

DMRT	Fr Dr	N	
A	22.2	12	128 F 20 s
A B	17.5	12	132 F 10 s
B	14.6	12	132 F 1 s
C	7.9	12	Untreated

Davis

Valencia/Swingle trees 10 years old. All 24 trees were steam treated on 4/9/15 at 120 F for 30 s. Canopy growth, fruit drop, and visible disease index (DI) have been monitored monthly since April 2015. On 6-22-16, half the trees were retreated with steam at 120 F for 30 s. On 9-22-16 and 12-12-16, Canopy Volume (CV), tree height (TH) and trunk cross sectional area (TCSA) were not affected by treatments. Disease index (DI) on not different on 9-22-16 but by 12-12-16, the DI of the untreated control trees increased and was higher than the DI of the trees that were retreated with steam at 120 F for 30 s. Data from Sept from previous reports have been included for reference. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Untreated Control				**TCSA**	
DATE	**N**	**CV (m^3)**	**TH (m)**	**(cm^2)**	**DI (0-40)**
9-22-16	12	19.4	2.3	123.9	17.6
12-12-16	12	23.2	2.7	124.8	19.3 a

Treatment = 120 F 30 s				**TCSA**	
DATE	**N**	**CV (m^3)**	**TH (m)**	**(cm^2)**	**DI (0-40)**
9-22-16	12	20.5	2.3	124.1	17.3
12-12-16	12	24.3	2.7	125.8	17.5 b

Shinn

Valencia /Swingle, 3 years old, double set. Pretreatment leaves were sampled for PCR on 8/7/2015. Eighteen trees were steam treated 8/7/2015 at 122-127 F (avg 125) for 30 s and 18 trees were untreated as controls. On 11-29-16, average Canopy Volume (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index (DI were unaffected by treatment. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

			11-29-16		
Treatment	**N**	**CV (m^3)**	**TH (m)**	**TCSA**	**DI (0-40**
Control	18	2.8	1.5	19.9	20.8
125 F 30 s	18	2.4	1.5	19.0	18.7

Lykes

Hamlin / X639 trees, 4 years old. 24 trees were steam treated on 10/6/15 at 55 C (131 F) for 30 s and 24 trees were left as untreated control trees. Canopy volume (CV, m^3), tree height (m), trunk cross sectional area (TCSA, cm^2) and disease index (0-40) were measured on Oct 10, 2016 and 12-7-16. Treated trees had smaller TCSA but had a lower DI (less visible disease) on Oct 10, 2016. By 12-7-16, however, the difference in DI had disappeared and the control trees had grown a larger CV and TCSA than the treated trees. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Treatment	N	10-10-16 CV (m^3)	12-7-16 CV (m^3)
Control	24	15.4	16.2 a
131 F 30 s	24	14.5 ns	14.3 b
		TH (m)	
Control	24	2.8	2.9
131 F 30 s	24	2.7 ns	2.9
		TCSA (cm^2)	
Control	24	75 a	75 a
131 F 30 s	24	63 b	63 b
		DI (m)	
Control	24	14.7 a	15.9 ns
131 F 30 s	24	13.6 b	15.2

Lee Jones

Based on initial PCR evaluations, 22 uniform trees that were HLB positive and 22 uniform HLB negative were selected for evaluation. On 3-22-16, one tree in each pair was steam treated at 128 F for 30 s while the other paired tree was left as an untreated control. On 10-12-16, canopy volume (CV), tree height (TH), trunk cross sectional area (TCSA) and Disease index were unaffected by treatment. Average fruit drop (Fr Dr), however, was lower in the steam treated trees. Leaves have been sampled for PCR analysis and await analyses. Evaluation of canopy volume, Disease Index and fruit yield have been scheduled in the first quarter of 2017.

Untreated Control

DATE	N	CV (m^3)	TH (m)	TCSA (cm^2)	DI (0-40)	Fr Dr
10-12-16	22	7.1 ns	2.4 ns	26.0 ns	13.1 ns	10.2 a

Treatment = 128 F 30 s

DATE	N	CV (m^3)	TH (m)	TCSA	DI	Fr Dr
10-12-16	22	7.5	2.5	27.5	13.6	5.3 b

CRDF Commercial Product Delivery Project Progress Report FY 2016-17

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2. Asian Citrus Psyllid VECTOR INTERVENTION

Project Title: 2a. Asian Citrus Psyllid Management and Citrus Health Management Areas (CHMAs)

Narrative of Progress by Project Goals:

1. Pursue actions that will support expanded tools for ACP management
   The continuing CRDF ACP portfolio has a number of active projects. Among them are projects that could may contribute to addressing the increased ACP pressure this year. Discussion with Project PI's identified that issues related to increased ACP populations, including fewer applications per grove and fewer groves being treated, questions regarding development of resistance, and performance of individual products. Current review of use of pyrethroids by EPA also is an issue of importance to continuing use of diverse classes of pesticides. Progress reports for this quarter outlining progress on several ACP projects were not available.

2. Engage registrants and regulatory entities in need for label modifications
   Several discussions were held in the 2nd quarter FY 2016-17 regarding the potential for Aldicarb pesticide to be considered for use in Florida citrus. A registrant has resumed manufacture and has marketed the product in other states on other crops. Discussions with the registrant and distributor has identified a series of questions and issues that surround re-introduction of Aldicarb into Florida citrus. Plans to determine the need for field research in 2017 are being discussed.

3. Continue participation in pesticide stewardship activities
   15-038C Resistance Monitoring: Dr. Stelinski continues to monitor at locations around the state for resistance development. Discussion among the researchers and growers have highlighted the importance of rotation of active ingredients. This topic is becoming more contested as growers are attempting to reduce ACP suppression costs and resort to lower cost spray materials.
   15-036C Distribution and behavior of pesticides targeting ACP: Correlating pesticide residue analysis with psyllid feeding to improve protection of young trees is providing results that will improve our understanding of the movement and retention of pesticides on/in targets and inform adjustments to spray recommendations, both timing and choice of materials. Portfolio review in Q3 will include identifying needs for moving this project into the next phase, and a broader approach has been suggested to meet the current needs.

4. Continue to support CHMA implementation of ACP and other HLB management tools
   CHMA meetings, further emphasis on APC suppression are continuing under leadership of Brandon Page and Dr. Rogers. These meetings addressed the increase in ACP populations in mid-summer cycles of CHRP scouting.
   The third phase of FDACS abandoned citrus grove removal is planned for Q3, with potential sites identified and agreements with FDACS being completed. This third phase will involve up to about

15,000 acres of unmanaged citrus, and should result in local impacts on ACP populations. FDACS plans for Phase 3 of this effort will deploy the state funding commitment to eliminate further acreage of abandoned groves. This state funding program will follow what has been done in recent years with MAC funding, but allows incorporation of ACP suppression to be conducted by the contractors prior to removing the acreage. All groves in this phase 3 program will be completed by end of fiscal year, June 30, 2017.

CRDF plans to coordinate evaluation of herbicides for disabling unmanaged groves from serving as ACP and CLas reservoirs. During this quarter, communications between the registrants, Third Party Registrations, FDACS Pesticides Division, and UF, IFAS were conducted and a draft plan was completed for a spring, 2017 field demonstration of the use of the herbicide.

5. Communicate progress and results of project to CPDC, CRDF and growers

Significant Meetings and Conferences:

None reported in Florida, but CRDF representations and many citrus researchers funded by CRDF participated in the September, 2016 International Citrus Congress in Brazil and also in the September International Congress of Entomology in Orlando, Florida. A large number of oral presentations, symposia, and posters were on the agenda, updating ongoing research related to management of ACP in the context of HLB management.

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2. ASIAN CITRUS PSYLLID VECTOR INTERVENTION

Project Title: 2b. RNAi Molecules/Psyllid Shield

Narrative of Progress against Goals:

Obj. 1 - Complete planning for and initiate field trials to begin in spring 2017

The principal goals of the 3- year field trials are as follows:

- Determine if selected target sequences that were found effective in a greenhouse environment against ACP when expressed in plants using the Citrus Trestiza Viral Vector (CTVvv) are effective in controlling ACP when delivered by CTVvv under field conditions
- Familiarize the regulatory agencies with the technologies and help establish the field testing conditions for trials with RNAi. This will enable the industry to help develop the testing protocols and permit conditions for testing in conjunction with the agencies instead of having the conditions established completely by the agencies or by others.
- Based on the results of this field trial, a decision will be made regarding a Phase 2 area-wide "Psyllid Shield" field trial.

During the quarter the following activities were performed:

- A research manager of the RNAi trials was hired by Southern Gardens out of Pioneer Hybrids, where he led projects and managed several research sites in Puerto Rico and California.
- All contractual agreements between CRDF and Southern Gardens were completed, effective September 1, 2016, in which CRDF will fund a portion of the direct expenses related to the trial, and Southern Gardens/Southern Gardens Nursery will provide in-kind funding to cover some direct and all indirect costs of the trial.
- In January hired the Research Technician who brings a background in insect rearing, trapping and cage design.
- Dr. Bill Dawson's lab (IFAS CREC) has been asked to begin to create each construct within the CTVvv technology.
- USDA and EPA permitting are in their final stages.

Southern Gardens team and CRDF were working to determine the ownership of the 6 constructs. This is essential for developing an MTA with the rightful owners of the technology.

Current plans are to plant in mid-to late spring.

Obj. 2 - Continue outreach to other companies engaged in RNAi research and product development for potential collaborations

This is an ongoing effort. During the quarter, efforts continued to identify and explore opportunities for collaboration with companies investing in RNAi research and development for use in agriculture.

Nothing new to report regarding on-going communications are continuing with Forrest Innovations, which is developing RNAi products to help shore up the defense mechanisms within citrus trees to

modulate the effects of the HLB pathogen. The company is currently focused on its mosquito control product due to the Zika outbreak, so progress has been slow.

During the quarter, contact was initiated with AUM LifeTech, an early stage company based in Philadelphia, that is working with USDA ARS to evaluate the efficacy of FANA RNA inhibition technology to control CLas. The goal is to learn more about the company and its technology, the status of development, and determine whether and how CRDF might be of assistance

Obj 3 - Continue to monitor ongoing RNAi research, including nuPsyllid project, for insights that may be applied to ACP intervention through Psyllid Shield.

This is an ongoing effort. As the nuPsyllid project progresses, there are an increasing number of insights that may be useful in implementing the Psyllid Shield concept of wide area deployment of "altered" psyllids with reduced capability to spread HLB. At the November 2016 CRDF Board meeting, there was discussion of the nuPsyllid project, and, at the request of the Board, Dr. Turpen is developing a white paper to communicate the expected outcomes from the nuPsyllid project as it comes to conclusion, as well as how the progress will be continued.

Obj 4 - Continue to explore potential candidates for long term commercialization of RNAi solutions for ACP intervention

This is an ongoing effort. CRDF continues to facilitate, accelerate and incentivize corporate action and is prepared to provide regulatory, commercial delivery and other support, as appropriate, to candidate partners.

Commercial partners will be needed for follow-on work to the phase one field trial described above This includes support for a Phase 2 area wide "Psyllid Shield" field trial, as well as supporting regulatory, product development and other work needed to bring products to market.

Past discussions with University of Florida Office of Technology Licensing (Dr. Byatt) and outreach to potential partners suggests the most likely commercial partners will come from within the citrus industry due to the relatively limited size of the market and opportunities for return on investment.

Significant Meetings or Conferences:

None.

Obstacles Encountered and Breakthroughs:

The key obstacle, as described in Obj 1, is to determine ownership of the 6 constructs to be used in the RNAi field trials.

Other Information:

This project has effectively combined the results of RNAi research into psyllid control with Psyllid Shield modeling to create the information needed to develop the recommendation to proceed with a two-phased field trial approach. It is expected that enough data would be available by the end of year 2 to make some educated guesses as to the effectiveness of the RNAi constructs to begin planning for larger scale trials. The larger scale field trials would be designed to further validate the technology and to collect the data necessary for a full section 3 registration.

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3. Citrus Host Intervention

Project Title: 3a. Naturally Occurring Microbial Product Interactions with HLB

Project goal(s)

1. Track ongoing research on soil microbes and their role in HLB and tree health 2. Conduct field trials to test commercially available naturally occurring microbes 3. Provide communication on project goals, progress and results to CPDC, CRDF and growers

Narrative of Progress by Project Goals:

2. Conduct field trials to test commercially available naturally occurring microbes.
The overall goal of the project is to screen candidate antimicrobials and deliver best performers through field trials to commercial use. This study is a side-by-side comparison of these 5 soil-applied commercially available products (+water control) as well as organic mulch as recommended by growers. We are testing the **hypothesis** that soil-applied products will mitigate the effects of HLB on tree health and yield. We expect that differentiation in tree health and disease status will appear in year 2, and after 3 years, we will have valid information on the true impact of these treatments on tree health, disease rating, HLB status, foliar nutrition, root density, yield and fruit quality.

Experimental protocols were developed to provide a sound scientific assessment of HLB effects of 5 commercially available microbial soil amendment products (BioFlourish, Ecofriendly, Serenade, Quantum and Aliette) plus a water treated control (UTC), in multiple applications per year as recommended. A subset of trees within each treatment was mulched with mature cow manure.

Ongoing treatments (quarterly or monthly) were began in May/June 2014 and are being applied with and without an organic mulch at the 3 Valencia/Swingle trial sites, **Ridge, East Coast, Southwest Florida**. All required field work at all 3 sites is on schedule and all the data has been submitted on time.

The Field Trial Project Manager, the Field Trial Administrator and Staff are monitoring the project activities. CRDF established data repositories for each project site so that all photos, data and treatment data are provided to CRDF as they are collected. Each of the 3 trials consists of the 6 treatments of 20 trees, 4 reps = 24 plots of 20 trees = 480 trees at each site plus. Sub-plots of 3 trees within each of the 24 plots = 72 trees mulched at each site. There are 6 unmulched sentinel trees in each treatment, replicated 4 times = 24 trees per treatment plus 1 mulched sentinel tree in each treatment replicated 4 times = 4 mulched trees per treatment. Thus, there are 28 sentinel trees times 6 treatments = 168 total trees at each site.

Contracted crop consultants are applying product treatments plus mulch, monitoring canopy volume and Decline Index (DI), photographing sentinel trees and taking leaf samples for PCR and nutrient analysis. At harvest, total fruit weight fruit is evaluated and samples are taken for juice quality analysis. Soil cores were sampled in Dec 2014, 2015 and 2016 to determine root density. Soil amendment effects

on root densities were not remarkable in 2014 and 2016 but were summarized in the June 2016 report. Root density data from 2016 have not been analyzed yet. This quarterly report (Dec 2016) focuses on recent Disease index ratings from the Ridge and East Coast sites along with leaf nutrient values from the SW Fl (Duda) site taken in Sept 2016, after 28 months of treatments.

Site Results to Date:

Ridge Site, Balm FL: Valencia/Swingle trees are 17 years old. After 2 years of treatments, there were no effects of the mulch treatment so + an – mulch treatments were combined for n=28 hereafter. There were no treatment effects on DI but the BioFlourish treatment resulted in larger canopy volumes than the Quantum and untreated control treatments. From the Jun 2016 report, there were no treatment effects on CT values nor on fruit yield from the April 27, 2016 harvest. All trees were HLB positive having a CT less than 31. Fruit yields were relatively low (1-1.2 boxes) for 17-year-old trees with canopy volumes of 29-41 m^3.

Leaves were sampled for nutrition analyses on 8-2-16, brought to the lab, washed, oven dried and sent to the IFAS commercial lab for analyses. Leaf samples from the 6 sentinel trees within each of the 4 replicate blocks per treatment were pooled (n=4 for each treatment) plus a separate sample from the mulched trees in each block for a total of N = 8 for each treatment. There were no treatment effects on leaf N, P or K but leaves from Untreated control and Bioflourish treated trees had lower Cu than the other treatments. There were no treatment effects on all other leaf nutrients measured as all others were at or near the optimum ranges for each element. There was no mulch effect on any measured nutrient. These data were in the Sept 2016 quarterly report. There was no mulch effect on any measured nutrient.

On 11/29/16, Disease Index (DI) was evaluated on all 168 sentinel trees. There were no mulch effects (data not shown) so plus and minus mulch treatments were combined yielding 6 trees x 4 reps plus 4 mulched trees for n=28 trees per treatment. The untreated control (UT Cont) treatment had the lowest numerical average DI rating but only the Ecofriendly treated trees had significantly higher DI than the untreated control. All the other treatments did not differ.

11_29_16
Disease Index (DI, 0-40)

DMRT		DI	N	Treatment
	A	10.8	28	EcoFriendly
B	A	9.8	28	Serenade
B	A	9.6	28	Aliette
B	A	9.5	28	Quantum
B	A	9.5	28	BioFlourish
B		7.8	28	UT Cont

Monthly or quarterly treatments have continued through 2016 and will continue through harvest 2017. Root samples and leaves samples for PCR analysis have been collected and await analyses. Canopy volume, Disease Index and fruit Yield have been scheduled in the first quarter of 2017.

East coast, Indian River site: Valencia/Swingle trees are 6 years old. From the Jun 2016 report, the BioFlourish and Ecofriendly treated trees had a lower DI 10, so looked better) than Untreated Control trees (DI=15). There were no treatment effects however, on CV, CT or fruit yield which averaged 1.3 boxes. All trees were HLB positive having a CT less than 31 (24-26).

Leaves were sampled for nutrition analyses on 8-8-16, brought to the lab, washed, oven dried and sent to the IFAS commercial lab for analyses. Leaf samples from the 6 measurement trees within each of the 4 replicate blocks per treatment were pooled (n=4 for each treatment) plus a separate sample from the mulched trees in each block for a total of N = 8 for each treatment. As described in the Sept 2017 quarterly report, all leaf nutrients were near or within the optimum range (on a % dr wt basis). Leaves from the Aliette treated trees had the highest leaf N (2.6%) and the other treatments did not differ. Leaf P was not affected by treatment but leaves from the Bioflourish and Aliette treatments had higher K than the other treatments. No other leaf nutrients were affected by treatments. Although well within the optimum range, the mulch treatment significantly increased leaf P by 0.01 %.

On 9/14 and 12/16/16, Disease Index (DI) was evaluated on all 168 sentinel trees. There were no mulch effects on DI in Sept or Dec (data not shown) so plus and minus mulch treatments were combined yielding 6 trees x 4 reps plus 4 mulched trees for n=28 trees per treatment. On 9/14/16, the Untreated (UT) Control trees had the highest DI but only the Serenade and Bioflourish trees had significantly lower DI. By 12/16/16, the DI of the Quantum, UT Control and Serenade treated trees significantly decreased to a DI below 15. Lumping the DI from the 2 months (n=56), the Serenade treated trees had the lowest average DI (13.7) versus the other 6 treatments which did not differ and averaged 15.5 (data not shown).

9_14_16
Disease index (DI, 0-40)

DMRT			DI	N	Treatment
	A		16.8	28	UT Control
B	A		16.7	28	Quantum
B	A	C	16.3	28	Aliette
B	A	C	16.3	28	EcoFrien
B		C	15.2	28	Serenade
		C	15.0	28	BioFlourish

12_16_16
Disease index (DI, 0-40)

DMRT			DI	N	Treatment
	A		16.1	28	BioFlourish
B	A		15.1	28	Aliette
B	A		15.0	28	EcoFrien
B	A		14.9	28	Quantum
B	C		13.7	28	UT Control
	C		12.2	28	Serenade

A grower field day was held on Dec 6, 2016 to highlight non-significant treatment effects on leaf nutrition, canopy volume and fruit yield at the East coast (Indian river) site. There were 42 people in attendance from all over the State.

Monthly or quarterly treatments have continued through 2016 and will continue through harvest 2017. Root samples and leaves samples for PCR analysis have been collected and await analyses. Canopy volume, Disease Index and fruit Yield have been scheduled in the first quarter of 2017.

SW FL Duda site: Valencia/Swingle trees are 11 years old. From the Jun 2016 report, there were a few differences in DI among treatments such that none of the average DI differed significantly from that of the untreated control. Canopy volumes of the BioFlourish, Aliette, Quantum and Ecofriendly treatments were all greater than Untreated Water Control and the Serenade treatment. None of the CT values from the treatments differed from the Water control but only the Serenade treated trees would be considered HLB + as having a CT lower than 31. Fruit yield averaged 3.2 boxes and corresponded to canopy size as the smaller Water control and Serenade treated trees had lower yields than the larger trees from the other 4 treatments. There were no treatment effects on total brix in the juice or on lb solids per box.

Leaves were sampled for nutrition analyses on 9-1-16, brought to the lab, washed, oven dried and sent to the IFAS commercial lab for analyses. Leaf samples from the 6 measurement trees within each of the 4 replicate blocks per treatment were pooled (n = 4 for each treatment) plus a separate sample from the mulched trees in each block for a total of N = 8 for each treatment.
These data were not yet analyzed for the Sept 2016 report so will be describes here.

There were no treatment effects on % leaf N as all were above the optimal range and not different from the water control. Although all trees had leaf P and K within the optimum ranges, Bioflourish treated trees had lower leaf P than the other treatment trees. In addition, Serenade treated trees had lower leaf K than the other treatment trees and did not differ from the water control.

N_% (Opt. = 2.5-2.8)

DMRT	% N	N	Soil Trt
A	3.1	8	BioFluor
A	3.0	8	Water
A	3.0	8	EcoFriend
A	2.9	8	Aliette
A	2.9	8	Serenade
A	2.8	8	Quantum

P_% (Opt. = .12-.17)

DMRT		% P	N	Soil Trt
	A	0.17	8	Serenade
	A	0.17	8	Water
B	A	0.17	8	Aliette
B	A	0.17	8	Quantum
B	A	0.16	8	EcoFriend
B		0.15	8	BioFluor

K_% (Opt. = 1.2-1.7)

DMRT		% K	N	Soil Trt
	A	1.8	8	EcoFriend
	A	1.7	8	Quantum
	A	1.7	8	Aliette
B	A	1.6	8	Water
B	A	1.6	8	BioFluor
B		1.5	8	Serenade

Only Aliette treated trees had higher leaf Cu than the water controls. Bioflourish and Alliette treated trees had lower leaf B than the other treatments except Ecofriendly and Bioflourish had equal leaf B. There were no other soil treatment effects on leaf nutrients (data not shown).

Cu_ppm

DMRT		Cu	N	Soil Trt
	A	241	8	Aliette
B	A	216	8	Serenade
B	A	204	8	EcoFriend
B	A	173	8	Quantum
B	A	163	8	BioFluor
B		138	8	Water

B_ppm

DMRT		B	N	Soil Trt
	A	69	8	Serenade
	A	69	8	Water
	A	69	8	Quantum
B	A	64	8	EcoFriend
B	C	58	8	BioFluor
	C	57	8	Aliette

Mulch effects: Lumping the 4 mulched trees across the 6 soil treatments yields n=24. Regardless of the soil treatments, the mulch treatment significantly increase leaf N, Mn and Zn above that in the unmulched trees. All other leaf nutrients were unaffected by the mulch treatment (data not shown).

N_% dw

DMRT	% N	N	Mulch
A	3.1	24	yes
B	2.8	24	no

Mn_ppm

DMRT	Mn	N	Mulch
A	82	24	yes
B	74	24	no

Zn_ppm

DMRT	Zn	N	Mulch
A	85	24	yes
B	78	24	no

Monthly or quarterly treatments have continued through 2016 and will continue through harvest 2017. Root samples and leaves samples for PCR analysis have been collected and await analyses. Canopy volume, Disease Index and fruit Yield have been scheduled in the first quarter of 2017.

3. Provide communication on project goals, progress and results to CPDC, CRDF and growers
A grower field day is scheduled for Mar 22, 2017 to highlight treatment effects at the SW Fl (Duda) site.

Significant Meetings of Conferences:

A presentation entitled "Soil Microbial Product Interactions With HLB in Valencia/Swingle Trees Over Three Seasons at Three Contrasting Sites in Florida" will be presented at the 5th International Research Conference on Huanglongbing March 14-17, 2017 in Orlando. A similar presentation and publication will be presented at the 130th annual Florida State Horticulture meetings in Tampa in June 4-6, 2017.

Obstacles Encountered and Breakthroughs:

Results of these field sites are being regularly communicated to the Florida citrus industry by CRDF through written reports, a quarterly progress report to the Committees and Board of CRDF which is posted to the CRDF website, and through presentation at grower meeting as indicated below. Completion of the fruit harvest data collection and analysis in June 2016, allowed a comprehensive view of the value of these treatments over the first 2.5 years of the trial. A grower field day was held on Dec 6, 2016 at the east coast (IR) site and a grower field day is planned for Mar 22, 2017 at the SW Fl (Duda) site to summarize treatment effects on canopy volume, disease index, leaf nutrition and fruit yield after almost 3 years of treatments.

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3. Citrus HOST INTERVENTION

Project Title: 3b. Deployment of Disease Resistant or Tolerant Citrus Rootstocks and Scions

Narrative of Progress against Goals:

Obj. 1 - Track ongoing research projects evaluating emerging scion and rootstock genotypes for tolerance or resistance to HLB, citrus canker, and other diseases.

CRDF implemented a new annual report system through which researchers could better communicate the progress of their research, successes and obstacles. The system is new which may explain why the reports do not reveal much quantitative data. Staff will follow with individual researchers.

Obj. 2 - Cooperate in in-depth evaluation and planning exercises related to Florida (and the US) citrus breeding to better focus on HLB solutions and rapid evaluation and deployment of rootstocks and scions

Obj. 3 - Develop and implement plans for expanded management of tolerant and resistant citrus

Pipelines to create a common platform to evaluate, identify and advance the best performing HLB tolerant/resistant candidates are being developed. The current activity centers on finalizing a draft pipeline integrating the Core Citrus Transformation Facility and the Mature citrus Transformation facility in the research pipeline.

Obj. 4 - Facilitate identification of best-performing candidate rootstocks and scions that appear to have HLB tolerance or resistance from Florida (and other) breeding programs

There has been some progress in the attempt to understand the plant improvement programs and how genotypes flow of genotypes through the conventional pipelines is conducted. However, this is only one out of three programs, so there remains quite a lot of information to be shared if a pipeline is to be generated to take advantage of the genetic variation generated by all programs.

There are two large-scale field trials in planning for both rootstock and scion candidate genotypes in Florida. Both of those trials are funded by the MAC projects and there is some uncertainty about funding for data collection. The rootstock trial will be planted this spring and there have been discussions around CRDF involvement and requests for resources for data collection. The scion trial is in planning stages and will likely not be ready for planting until the spring of 2018.

Obj. 5 - Implement and evaluate Phase I and II grower field trials of most promising candidate HLB tolerant rootstocks using standard varieties as scions.

Phase I field trials:

In the Summer of 2016, the first fruit set was stripped from two sites BHG (ridge) and Peace River (ridge) to promote vegetative growth. Total fruit weight and count data were collected and analyzed within the two locations. Due to the large fruit crop at the Duda (Southwest) site, fruit stripping was stopped after the first replicate, and the rest of the site will be harvested in late Spring 2017.

At all three sites, twenty 4-6-month-old leaf samples per plot were collected processed and sent for laboratory analysis for leaf nutrient content. Data received were analyzed and are presented for each site. Macronutrients are presented on a percent leaf dry weight basis while micronutrients are presented on a parts per million (ppm) basis. Calculations on a leaf area basis did not show any cause of concern of leaf nutrient over over estimation due to hyper accumulation of starch.

Field evaluations of field trials are ongoing using standardized CRDF protocols for evaluation and data collection of HLB diseases incidence and horticultural traits. During the fourth quarter of 2016 horticultural data tree height (cm), canopy volume (m^3) and trunk cross-sectional area (cm^2) were collected and analyzed for rootstock differences within each site. HLB disease index (DI) was rated on a maximum scale of 0 to 5 per side of the crown, with 0 denoting no visual symptoms and 5 severe decline on more than 80% of the canopy. The maximum possible score for DI in these trials is 10.

Data Analysis and Results

All sites are planted in a completely randomized design (CRD) with 5 replications per rootstock. Data were analyzed using a mixed model analysis procedure GLIMMIX of SAS (SAS institute Inc, 2004) with the appropriate comparisons to test for differences among rootstock means.

All the rootstock data collected was analyzed within each site and not compared across all sites. It will be important to compare rootstock performance across sites as the trials mature, especially when yield and fruit quality data become available. Current results suggest it is too early to make such a comparison, although one can be made retrospectively later.
Data analysis for the two ridge sites (BHG and Peace River) exclude UFR-16 which was planted late at both locations and cannot be fairly compared to the other rootstocks. Despite the two planting dates of UFR-3 inclusion or exclusion from data sets did not affect the results and so it was left in the data sets for analysis.

Results for data that has previously not been reported are presented by location.

CRDF DUDA Rootstock Trial, Felda, FL (Southwest)
The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance. All trees were planted in March 18,19, 2015. The rootstocks were US-812, US-942, UFR-2, UFR-3, UFR-4, UFR-16 and Swingle (as a standard). Eight sentinel trees were randomly assigned to each plot at planting for data collection.

Horticultural Trait Data

There were no significant differences ($p < 0.05$) in HLB DI among rootstocks or at this location (Table 1.). All the rootstocks were rated ~1 which indicates dense canopy with minimal blotchy mottle and no dieback.

Table 1. CRDF DUDA rootstock trial HLB DI collected in Fall 2016

Rootstock	HLB DI[a]
UFR_3	1.3 ± 0.1
US_942	1.2 ± 0.1
UFR_2	1.2 ± 0.1
SWINGLE	1.1 ± 0.1
US_812	1.1 ± 0.1
UFR_16	1.0 ± 0.1
UFR_4	0.8 ± 0.0

[a]HLB DI rating on a scale of 0 to 5 per side of the tree 0= no foliar symptoms, 5 = foliar symptoms on more than 80% of the canopy

There were significant differences ($p < 0.05$) among rootstocks for canopy volume (m^3), trunk cross-sectional area (cm^2) and tree height (cm), Tables 2, 3 and 4. The best performing rootstocks were US_942, US_812, and UFR_4 for all three horticultural traits evaluated this quarter while UFR-16, UFR_2 and UFR_3 had lower values. Swingle performance was generally midway between the experimental rootstocks.

Table 2. CRDF Duda rootstock trial canopy volume (m^3)

Rootstock	Canopy Volume (m^3)
US_942	7.11 ± 0.28 A
US_812	6.59 ± 0.23 A
UFR_4	6.54 ± 0.17 A
SWINGLE	5.56 ± 0.20 B
UFR_2	5.36 ± 0.18 B
UFR_16	5.12 ± 0.24 B
UFR_3	4.02 ± 0.22 C

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

There were significant differences ($p < 0.05$) among rootstocks for canopy volume (m^3), trunk cross-sectional area (cm^2) and tree height (cm), Tables 2, 3 and 4. The best performing rootstocks were US_942, US_812, and UFR_4 for all three horticultural traits evaluated this quarter while UFR-16, UFR_2 and UFR_3 had lower values. Swingle performance was generally midway between the experimental rootstocks.

Table 3. CRDF Duda rootstock trial trunk cross-sectional area (cm^2)

Rootstock	Trunk Cross Sectional Area (cm^2)
US_942	33.72 ± 0.92 A
US_812	31.95 ± 1.03 A
UFR_4	28.05 ± 0.64 B
SWINGLE	26.87 ± 0.72 BC
UFR_16	24.63 ± 1.01 BC
UFR_2	23.86 ± 0.63 CD
UFR_3	20.33 ± 1.16 D

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Table 4 CRDF Duda rootstock trial tree height (cm)

Rootstock	Tree Height (cm)
US_942	218.9 ± 3.7 A
UFR_4	211.3 ± 2.6 AB
US_812	205.1 ± 2.9 AB
SWINGLE	199.0 ± 4.0 BC
UFR_16	187.1 ± 3.8 C
UFR_2	186.1 ± 2.9 C
UFR_3	167.1 ± 4.1 D

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Leaf Nutrition data

There were significant ($p < 0.05$) differences in rootstocks for all leaf tissue macronutrients (percent dry weight) except nitrogen, although the mean comparison is not remarkable (Table 5). Data in Table 5 is sorted by rootstock with the standard at the top rather than by nutrient content. Macronutrient amounts within rootstock generally fall within the minimum guidelines for citrus (IFAS Bulletin, http://edis.ifas.ufl.edu/pdffiles/ss/ss47800.pdf).

Table 5. CRDF Duda rootstock leaf tissue macronutrient content (percent dry weight)

Rootstock	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
Swingle	1.83 ± 0.05	0.15 ± 0.00 AB	1.19 ± 0.12 AB	3.89 ± 0.21 ABC	0.26 ± 0.01 B
UFR_16	1.70 ± 0.11	0.13 ± 0.00 B	1.14 ± 0.13 AB	4.51 ± 0.08 A	0.35 ± 0.02 A
UFR_2	1.60 ± 0.08	0.17 ± 0.01 AB	1.08 ± 0.14 AB	3.29 ± 0.32 BC	0.29 ± 0.01 AB
UFR_3	1.86 ± 0.13	0.17 ± 0.01 AB	1.35 ± 0.09 A	2.90 ± 0.10 C	0.27 ± 0.00 B
UFR_4	1.88 ± 0.04	0.19 ± 0.00 A	1.00 ± 0.07 AB	3.37 ± 0.09 BC	0.35 ± 0.01 A
US_812	1.65 ± 0.04	0.14 ± 0.01 AB	0.94 ± 0.12 B	4.31 ± 0.33 AB	0.30 ± 0.01 AB
US_942	1.84 ± 0.05	0.15 ± 0.01 AB	0.85 ± 0.09 B	4.10 ± 0.35 AB	0.24 ± 0.00 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Leaf tissue micronutrient content (ppm on a dry weight basis) among all the rootstocks lie within the general production guidelines, with the exception of iron (Table 6). Although, there were significant rootstock effects for iron, zinc, copper and boron ($p < 0.05$), it is difficult to draw conclusions about the relationship between the rootstock and nutrients at this time. There was no significant effect of rootstocks on manganese content.

Table 6. CRDF Duda rootstock leaf tissue micronutrient content (ppm on dry weight basis)

Rootstock	Iron (ppm)	Manganese (ppm)	Zinc (ppm)	Copper (ppm)	Boron (ppm)
Swingle	42.29 ± 3.21 AB	34.51 ± 3.93	26.75 ± 3.65 AB	38.38 ± 5.05 AB	68.05 ± 1.78 B
UFR_16	35.96 ± 5.06 B	36.26 ± 4.82	29.20 ± 5.07 AB	38.53 ± 4.30 AB	72.79 ± 4.47 AB
UFR_2	39.87 ± 4.91 AB	30.55 ± 3.20	18.86 ± 4.55 AB	28.72 ± 6.69 AB	67.82 ± 2.27 B
UFR_3	37.59 ± 3.34 B	39.65 ± 7.04	34.41 ± 6.49 A	43.46 ± 6.51 A	85.60 ± 3.88 A
UFR_4	50.08 ± 6.40 AB	29.29 ± 5.35	18.27 ± 5.12 AB	15.73 ± 3.64 B	75.00 ± 4.89 AB
US_812	61.39 ± 6.93 A	28.03 ± 4.47	17.95 ± 4.21 AB	29.09 ± 3.71 AB	72.56 ± 3.19 AB
US_942	44.73 ± 5.86 AB	23.22 ± 4.97	16.03 ± 5.54 B	25.40 ± 6.88 AB	71.61 ± 4.40 AB

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Peace River CRDF Rootstock Trial, Babson Park, FL (Ridge)

The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance.Valencia trees on seven of eight rootstocks (US-897, US-942, US-812, UFR-2, UFR-4, UFR-3 (short half of the trees), & Carrizo (as a standard) were planted in April, 2015. Planting of UFR-3 trees was completed in September 2015. Trees on UFR-16 were planted in August 2016. Eight sentinel trees were randomly assigned to each plot at planting for data collection.

Horticultural Trait Data

There were significant differences ($p < 0.05$) for all horticultural traits reported for this period at the peace river location for DI, canopy volume (m3), trunk crossectional area (cm2), and tree height, tables 7, 8, 9 and 10, respectively. The comparisons of mean DI show two groups which have a low incidence of symptoms (DI < 1) (Table 7). Canopy volume(m^3), trunk cross-sectional area (cm^2) and tree height data (cm) show that trees on US_942 are larger than the other rootstocks with UFR_2 and UFR_3 having much smaller trees.

Table 7 *CRDF Peace River rootstock trial HLB DI collected in Fall 2016*

Rootstock	HLB DI[a]
UFR_2	0.9 ± 0.1 A
UFR_4	0.6 ± 0.1 AB
Carrizo	0.5 ± 0.1 AB
US_812	0.5 ± 0.1 AB
UFR_3	0.4 ± 0.1 B
US_897	0.3 ± 0.1 B
US_942	0.3 ± 0.1 B

[a]HLB DI rating on a scale of 0 to 5 per side of the tree 0= no foliar symptoms, 5 = foliar symptoms on more than 80% of the canopy

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Table 8 CRDF Peace River rootstock trial canopy volume (m^3)

Rootstock	Canopy volume (m^3)
US_942	2.56 ± 0.11 A
US_812	2.12 ± 0.10 AB
UFR_4	1.96 ± 0.14 B
US_897	1.69 ± 0.10 BC
Carrizo	1.38 ± 0.12 CD
UFR_2	0.95 ± 0.09 D
UFR_3	0.37 ± 0.10 E

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Table 9 CRDF Peace River rootstock trial trunk cross sectional area (cm^2)

Rootstock	Trunk cross-sectional area (cm^2)
US_942	15.37 ± 2.71 A
Carrizo	10.81 ± 4.25 B
UFR_4	10.74 ± 2.95 B
US_812	12.44 ± 2.34 B
US_897	10.66 ± 3.04 B
UFR_2	6.89 ± 2.69 C
UFR_3	3.95 ± 3.84 D

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

Table 10 CRDF Peace River rootstock trial tree height (cm)

Rootstock	Tree Height (cm)
US_942	158.2 ± 2.6 A
US_812	153.6 ± 2.8 A
UFR_4	145.4 ± 2.9 AB
US_897	147.0 ± 3.0 AB
Carrizo	137.1 ± 3.9 BC
UFR_2	128.5 ± 2.9 C
UFR_3	110.9 ± 3.9 D

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Fruit were stripped from all the plots at Peace River in June of 2016 to promote vegetative growth. Staff collected total fruit weight and counts which may be used as a measure of precocity. There were significant effects of the rootstocks ($p < 0.05$) on total fruit weight and pieces of fruit counted (Table 11). Fruit weight and count are consistent with tree size with the largest trees having the greatest estimated measure of precocity.

Table 11 Peace River total fruit weight (Kg) and fruit count (pieces) stripped in June 2016

Rootstock	Total Fruit Weight (Kg)	Fruit Count (pieces)
US_942	23.36 ± 2.31 A	414.2 ± 44.8 A
US_897	13.24 ± 1.57 B	246.0 ± 24.0 AB
Carrizo	7.02 ± 1.44 BC	142.4 ± 30.5 B
UFR_3	5.08 ± 1.97 BC	135.6 ± 51.8 B
UFR_4	6.88 ± 1.70 BC	162.0 ± 45.8 B
US_812	8.72 ± 1.91 BC	182.6 ± 41.2 B
UFR_2	3.50 ± 2.09 C	79.8 ± 42.9 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Leaf Nutrition Data

There was a significant ($p < 0.05$) effect of the rootstocks on leaf tissue macronutrients except phosphorus and calcium, although the the mean comparisons of the other macronutrients do not reveal a wide range of values (Table 12). Nitrogen and calcium levels fell just under the optimum rates, 2.5 – 2.7 % and 3 -4.9%, respectively. Macronutrient amounts within rootstock generally fall within the minimum guidelines for citrus (IFAS Bulletin,

http://edis.ifas.ufl.edu/pdffiles/ss/ss47800.pdf)

Table 12 CRDF Peace River rootstock trial leaf tissue macronutrient content (percent dry weight)

Rootstock	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
Carrizo	1.64 ± 1.64 B	0.16 ± 0.01	1.43 ± 1.45 AB	2.28 ± 0.39	0.33 ± 0.02 A
UFR_2	1.88 ± 1.88 B	0.17 ± 0.02	1.53 ± 1.55 AB	2.07 ± 0.27	0.33 ± 0.02 A
UFR_3	2.41 ± 2.41 A	0.18 ± 0.01	1.67 ± 1.69 A	1.72 ± 0.64	0.27 ± 0.02 AB
UFR_4	1.87 ± 1.87 B	0.18 ± 0.03	1.25 ± 1.27 B	2.19 ± 0.22	0.34 ± 0.01 A
US_812	1.88 ± 1.88 B	0.16 ± 0.01	1.50 ± 1.53 AB	2.44 ± 0.44	0.28 ± 0.00 AB
US_897	1.93 ± 1.93 AB	0.19 ± 0.03	1.22 ± 1.24 B	2.19 ± 0.63	0.26 ± 0.00 AB
US_942	1.97 ± 1.97 AB	0.15 ± 0.01	1.36 ± 1.38 AB	2.55 ± 0.34	0.25 ± 0.01 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

There were significant variations in leaf tissue micronutrient content among the rootstocks at the Peace River site for all micronutrients except boron (Table 13). Leaf tissue content was lower than the optimum recommended ranges for iron (60- 120 ppm) and boron (36-100ppm).

Table 13 CRDF Peace River rootstock trial leaf tissue micronutrient content (ppm dry weight)

Rootstock	Iron	Manganese	Zinc	Copper	Boron
Carrizo	35.53 ± 7.15 AB	61.05 ± 9.99 B	78.01 ± 5.78 B	10.11 ± 2.07 AB	14.92 ± 1.09
UFR_2	36.47 ± 7.77 AB	77.40 ± 12.93 AB	83.24 ± 2.83 B	7.24 ± 0.68 AB	14.07 ± 1.82
UFR_3	39.11 ± 5.79 AB	112.34 ± 42.73 A	120.5 ± 23.97 A	12.70 ± 2.37 A	48.04 ± 21.15
UFR_4	27.79 ± 2.27 B	67.98 ± 22.74 B	93.96 ± 22.95 AB	3.06 ± 1.37 B	17.37 ± 1.00
US_812	59.29 ± 7.92 A	54.03 ± 8.05 B	66.32 ± 11.10 B	7.15 ± 0.54 AB	15.90 ± 1.57
US_897	42.07 ± 7.92 AB	59.79 ± 15.61 B	71.24 ± 21.84 B	5.86 ± 2.41 AB	15.08 ± 1.32
US_942	38.99 ± 3.23 AB	64.77 ± 7.16 B	76.07 ± 9.46 B	7.44 ± 1.52 AB	13.47 ± 0.79

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level.

BHG CRDF Rootstock Trial, Venus, FL (Ridge).

The trial is planted in a completely randomized design (CRD) with five replications of each rootstock budded with '1-14-19 Valencia' for straight comparison of rootstock performance. Eight sentinel trees were randomly assigned to each plot at planting for data collection. Valencia trees on 5 of 7 rootstocks were planted July 2015. Only trees on 5 rootstocks were initially planted: UFR-2, UFR-4, US-942, US-812 and Sour orange as a standard. Trees on UFR-3 were planted in September 2015 and trees on UFR-16 were planted in June 2016.

Horticultural Trait Data

There was no significant ($P < 0.05$) effect of rootstocks on HLB DI at the BHG site and all the trees have very low scores, and many (raw data not shown) had no visual symptoms (Table 14).

Table 14 CRDF Ben Hill Griffin rootstock trial HLB DI collected in Fall 2016

Rootstock	HLB DI[a]
US_812	0.4 ± 0.1 A
US_942	0.4 ± 0.0 A
UFR_3	0.4 ± 0.0 A
SOUR	0.4 ± 0.0 A
UFR_4	0.3 ± 0.0 A
UFR_2	0.2 ± 0.0 A

[a]*HLB DI rating on a scale of 0 to 5 per side of the tree 0= no foliar symptoms, 5 = foliar symptoms on more than 80% of the canopy*

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Rootstock effects were significant ($p < 0.05$) for canopy volume, trunk cross-sectional area and tree height ,Tables 15, 16 and 17, respectively. As with the data presented for the Duda and Peace River sites, post hoc comparisons of means were performed using the Tukey-Kramer method except for trunk cross-sectional area at BHG where Fisher's LSD ($p < 0.05$) was used to get a clearer separation among rootstocks.

Table 15 CRDF Ben Hill Griffin rootstock trial canopy volume (m^3)

Rootstock	Canopy Volume (m^3)
US_812	1.37 ± 0.05 A
US_942	1.32 ± 0.05 A
SOUR	1.04 ± 0.05 B
UFR_4	0.97 ± 0.04 B
UFR_2	0.66 ± 0.03 C
UFR_3	0.39 ± 0.02 D

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Table 16 CRDF Ben Hill Griffin rootstock trial trunk cross-sectional area (cm^2)

Rootstock	Trunk cross-sectional area (cm^2)
US_812	9.11 ± 0.30 A
US_942	9.58 ± 0.23 A
UFR_4	9.99 ± 4.19 A
SOUR	8.33 ± 0.26 A
UFR_2	5.34 ± 0.16 AB
UFR_3	3.17 ± 0.11 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Table 17 CRDF Ben Hill Griffin rootstock trial tree height (cm)

Rootstock	Tree Height (cm)
US_812	136.2 ± 2.4 A
US_942	134.0 ± 1.8 AB
SOUR	126.0 ± 2.1 BC
UFR_4	124.9 ± 1.9 C
UFR_2	115.5 ± 1.7 D
UFR_3	107.1 ± 1.7 E

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Canopy volume and tree height have a clear separation of rootstocks by size with US_812 being the largest tree. However, trunk crossectional area data have all the trees with similar performance with the exception UFR_3 which is the smallest. Further, examination of the trunk cross-sectional area data may be required to parse out the reasons for the observations.

The fruit was stripped from all the plots in July 2016 and fruit weight, and count data was collected for analysis and estimation of precocity. The effect of the rootstock was significant ($p < 0.05$) for both traits (Table 18).

Table 18 CRDF Ben Hill Griffin Fruit total fruit weight (Kg) and fruit count (pieces) stripped in July 2016

Rootstock	Total Fruit Weight (Kg)	Total Fruit Count
SOUR	6.61 ± 0.86 A	141.57 ± 17.01 A
US_942	4.90 ± 0.85 A	106.17 ± 16.53 A
US_812	2.70 ± 0.25 B	61.76 ± 6.49 B
UFR_2	2.26 ± 0.41 B	56.56 ± 9.91 B
UFR_4	2.16 ± 0.30 B	49.56 ± 6.54 B
UFR_3	1.46 ± 0.13 B	34.16 ± 2.99 B

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Rootstock effects were significant ($p < 0.05$) for total fruit weight (Kg) and total fruit count with Sour Orange and US-942 giving the highest estimate of precocity and no mean differences among the other rootstocks. It is interesting to note that US_812 has a low estimate of precocity despite having a larger canopy volume (Table 15).

Leaf Nutrition Data

There were no significant differences in nitrogen content among the rootstocks at the Ben Hill Griffin site (Table 19). While the effect of the rootstock on phosphorus, potassium, calcium and magnesium were significant, the pairwise comparisons of the means have a narrow range of differences. The calcium level was slightly below the optimum range (3 -4.9%).

Table 19 CRDF Ben Hill Griffin rootstock leaf tissue macronutrient content (percent dry weight)

Rootstock	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium
SOUR	2.51 ± 0.10	0.15 ± 0.00 BC	1.61 ± 0.01 B	2.30 ± 0.07 AB	0.28 ± 0.00 BC
UFR_2	2.41 ± 0.14	0.16 ± 0.00 ABC	1.55 ± 0.02 B	2.07 ± 0.08 B	0.33 ± 0.00 AB
UFR_3	2.70 ± 0.06	0.17 ± 0.00 A	2.24 ± 0.04 A	1.39 ± 0.11 C	0.28 ± 0.01 BC
UFR_4	2.49 ± 0.23	0.17 ± 0.00 AB	1.44 ± 0.03 B	2.08 ± 0.11 B	0.37 ± 0.01 A
US_812	2.45 ± 0.11	0.15 ± 0.00 C	1.59 ± 0.07 B	2.55 ± 0.13 A	0.32 ± 0.01 AB
US_942	2.58 ± 0.08	0.15 ± 0.00 ABC	1.53 ± 0.08 B	2.71 ± 0.06 A	0.25 ± 0.01 C

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

There were significant differences ($p < 0.05$) among rootstocks for all micronutrients except iron which was also lower than the recommended range (60 – 120 ppm). There was a wide range of values for manganese, Zinc, copper and boron which were well above published optimum values.

Table 20 CRDF Ben Hill Griffin rootstock leaf tissue micronutrient content (ppm on dry weight basis)

Rootstock	Iron	Manganese	Zinc	Copper	Boron
SOUR	37.31 ± 3.90	79.10 ± 4.26 C	32.87 ± 2.05 C	26.48 ± 3.06	44.87 ± 1.25 D
UFR_2	44.64 ± 3.57	123.76 ± 8.32 A	59.50 ± 5.35 AB	31.49 ± 4.11	65.60 ± 2.24 BC
UFR_3	41.82 ± 3.32	114.18 ± 7.97 AB	80.69 ± 4.17 A	33.09 ± 3.34	106.0 ± 5.59 A
UFR_4	43.43 ± 8.31	117.62 ± 8.34 AB	63.98 ± 7.93 AB	24.93 ± 1.79	70.94 ± 3.51 B
US_812	52.85 ± 3.97	92.38 ± 6.69 BC	44.33 ± 5.44 BC	23.42 ± 1.88	51.76 ± 1.98 CD
US_942	48.94 ± 3.09	89.45 ± 5.30 BC	51.08 ± 5.18 BC	27.72 ± 2.18	51.96 ± 4.31 CD

Values represent the mean ± standard error. Means were analyzed with a one-way ANOVA, and letter groupings were obtained using the Tukey-Kramer method. Values followed by the same letter do not differ significantly at the 5% level

Summary

Data presented for the three rootstock sites (Duda, Peace River and Ben Hill Griffin) are collected quarterly for horticultural traits (except yield) and annually for leave nutrients. We will continue to evaluate these trials this way until the data suggest a change to a biannual or annual evaluation of certain traits.

Obj. 6 - Communicate progress and results of evaluation of rootstocks to industry

A field day is in planning for the CRDF rootstock trials in March, 2017.

Significant Meetings or Conferences:

Obstacles Encountered and Breakthroughs:

Determining the status of plant improvement efforts by many researchers with different approaches and research philosophies is challenging. This challenge is further is underscored by reluctance in some to provide information which would further our understanding of progress and challenges encountered.

Other Information:

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Quarter Ending December 31, 2016

3. CITRUS HOST INTERVENTION

Project Title: 3c. Genetic technology (MCTF): Deploying Canker-Resistant Genes

Project Goals for FY2016-2017

Make measurable progress toward producing transgenic citrus lines from mature tissue transformation of commercially available cultivars for the Florida citrus growers. These citrus lines will have disease resistance to citrus canker and HLB, and will flower and bear fruit in a short time period.

Narrative of Progress Against Goals:

Obj. 1a - Continue Agrobacterium and biolistic transformation with genes to confer disease tolerance to HLB and canker as a service

Productivity significantly decreased during the quarter after the move to the packing house while the AC in the lab was being repaired. There was biological contamination of cultures, presumably due to autoclave issues, unsealed windows, or poor temperature control. Bacterial and fungal clean tests of mature citrus budwood from the growth facility in LB and LW broth, respectively, showed that all mother trees were clean, even the new cultivar introductions (B770, OLL8, Vernia, red grapefruit). With the move back into the lab in early December, the lab is now targeting two agrobacterium transformations per week to make up for lost time.

As a result, the number of Agrobacterium transformations with disease resistant genes slowed significantly from the prior quarter, with only approximately 10 transgenics being produced, one of which did not survive micrografting. The results of the remainder are pending. In addition, 10 immature Swingle transgenics for Dr. Wang and Dr. Orbovic were micro-grafted due to issues in the Citrus Core Transformation Facility. One shoot died and the results on the others are pending.

Obj. 1b - Biolistics: progress will be made in optimizations for mature citrus scion

During the quarter, efforts to optimize the amount of DNA per shot, time of bombardment and helium pressure (psi) to coincide with organogenesis were limited due to the move to the packing house. The goal remains to complete optimization and minimal cassettes transfer by June 2018.

Obj. 1c - Determine which of the micro-grafting steps can be bypassed altogether by growing explants in bioreactors for elongation of shoots and secondary grafting

During the quarter, efforts to shorten the time involved in transgenic plant production were also limited due to the move to the packing house. The goal remains to achieve this objective by June 2018.

Obj. 1d - Compare genes thought to enhance shoot production/transformation efficiencies and apply pre-treatments to increase organogenesis in mature rootstock

During the quarter, the lab identified a cDNA that dramatically increases mature scion transformation efficiencies and are investigating whether it will increase efficiencies in all cultivars. An invention disclosure entitled "A Method to Increase Organogenesis and Transformation Efficiencies in Recalcitrant Woody Species Such as Mature Citrus" was submitted by Dr. Zale to UF/IFAS Office of Technology Licensing.

Obj. 1e - Determine efficiencies of PMI selection in biolistics and Agrobacterium-mediated transformation compared to nptII.

MCTF continued its investigation of whether Phosphomannose isomerase (PMI) selectable marker will be useful for mature citrus transformations. The focus was on manipulating mannose concentrations to determine impact on shoot regeneration. Different concentrations are required for shoot regeneration in mature vs immature citrus, and more sucrose is necessary for shoot development in scion than rootstock.

Obj. 2 - Test a more sensitive, non-destructive screening process to increase throughput

The current process uses a colorimetric substrate (GUS) histochemical assay that is labor intensive, tedious and destructive to tissue, and produces a visible blue stain as a marker. The lab has been evaluating a new screen that is more sensitive and less destructive, using fluorescent MUG as an alternative substrate to GUS for fluorometric detection.

The lab has set a goal of June 2017 to complete evaluation to determine if shoots survive the MUG application and subsequent grafting steps, and whether there will be auto-fluorescence in non-transformed shoots, i.e. false positives.

Obj. 3 - Test new breeder lines using standard tissue culture protocols to determine whether optimizations are necessary.

The facility continues the process of introducing new breeder lines in which to produce transgenics. Recent additions have included Kurhaski, a rootstock similar to Carrizzo but with some nemotode tolerance. It has also included Glen Naval sweet orange cultivar, which is pollen sterile, so it will provide a contained system to prevent transgene flow. These were provided to Drs. Grosser and Dutt through shoot tip grafting (STG). Mandarin and pummelo were also introduced for Dr. Wang.

Obj. 4 - Increase throughput of budded plants in the growth room

This remains a major team effort. Measures are being pursued such as increasing planting density using citrus pots where possible, and, after budding, leaving the bud stick attached to scion to accelerate growth.

Significant Meetings/Conferences/Publications

A manuscript (25% funded by CRDF and 75& by CRB) was submitted to PCTOC and is in review. Y. Acanda, M. Canton, H. Wu, and J. Zale. Kanamycin selection in bioreactors allows visual selection of transgenic citrus shoots.

Obstacles Encountered

There have been unanticipated growth room repair and maintenance expenditures during the last quarter. This included rebuilding the water softener, replacing AC ducts, and repairing the sprayer.

Breakthroughs

None

Other Information

In June 2016, CPDC and the CRDF Board approved a two-year project continuation. (Project 15-045C).

MCTF's mission is to develop protocols for mature transformation of citrus that can be used to incorporate genes of interest, when available, into Florida cultivars. Through MCTF, CREC will generate the first mature sweet orange transformants with development protocols adjusted in the lab and in the growth room for Valencia, Hamlin and other commercial cultivars.

MCTF remains an important element of the overall pipeline encompassing both conventional breeding and genetic transformation, from inception, to field testing, to scale-up and delivery to growers. MCTF's role in this overall process is tied to CRDF efforts address the overall process for HLB host resistance and tolerance, including side-by-side field testing of the most promising candidates and delivery to Florida growers.

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Quarter Ending December 30, 2016

4. Other Citrus Diseases

Project title: 4a. Post-Bloom Fruit Drop

Narrative of Progress against Goals:

Obj. 1 - Summarize grower experiences in suppressing PFD during 2016 epidemic year

A survey for data collection was developed to evaluate severity of PFD in groves and CRDF has since surveyed twenty-one blocks. Data was collected from twenty trees per site. Fruit and residual fruit calyx buttons within a 0.5 square meter frame was counted twice on each side of the tree (4x total) and information on PFD treatments was collected from the growers. The goal of this survey was to detect trends that led to more or less PFD in specific groves and identify effective treatments. In the end, no effective treatment could be identified because not enough data could be collected.

Obj. 2 - Evaluate PFD management tactics under field conditions

The ongoing project titles “Enhancement of postbloom fruit drop control measures” was initiated in March 2016. This project is evaluating the efficacy and economics of PFD treatments, evaluating the period of efficacy of Luna Sensation during flowering, and determining if the flowering period can be narrowed using plant growth regulators, to eliminate offseason bloom. Applications were made in the 2016 season and will continue in 2017. The PGR trials have been initiated, this objective was added in the second year of this project.